REMARKS

After entry of this amendment, Claims 1-3, 5-7, and 9-11 will be all the claims pending in the application. Claims 1, 3 and 9 have been amended. Claims 4 and 8 have been canceled. Claim 5 has been amended to depend from a non-canceled claim. Claims 10 and 11 are new.

Support for the amendment to Claim 1 may be found in the specification, e.g., at Example 3.

Support for the amendment to Claim 3 may be found in the specification, e.g., at page 3, lines 13-14, and at page 7, lines 4-9.

Support for the amendment to Claim 9 may be found in the specification, e.g., at page 7, lines 12-19.

Support for new Claim 10 may be found in the specification, e.g., at page 12, lines 24-26.

Support for new Claim 11 may be found in the specification, e.g., at page 6, lines 2-8.

Entry of the above amendments is respectfully requested.

Preliminary Matters

Applicants thank the Examiner for withdrawing the rejection under 35 U.S.C. § 112 of Claim 6.

Claim Rejections - 35 U.S.C. § 102

On page 5 of the Office Action, Claims 1-5 and 9 remain rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Goodwin et al. (U.S. Patent 5,496,722) and Schwarz et al. (U.S. Patent 5,026,650) with support from Unsworth et al.

Initially, Applicants submit that Claim 4 has been canceled thereby rendering the rejection moot for this claim.

Further, Applicants have amended Claim 1 as shown above. Applicants submit that Goodwin et al. do not disclose production of cartilage tissue from bone marrow cells. Instead, Goodwin et al. merely discloses a "three-dimensional structural tissue mass developed with functional chondrocytes and stromal cells" (see, column 6 lines 24-25) where "the tissue exhibited matrix deposition including cells immunochemically characterized as producing Type IV, Type IX and Type X collagen" (see, column 6 lines 26-28).

Type II collagen is the basis for articular cartilage and one of the typical cartilage markers. It makes up 50% of all protein in cartilage and 85-90% of the collagen of articular cartilage. Goodwin et al. do not disclose the production of Type II collagen, only the production of Type IV, IX, and X collagens (Column 6 lines 22-34).

Among cartilage markers such as Type II, IX, X, and XI collagens, Type II collagen is the most important marker to characterize cartilage. Type X collagen is a marker for hypertrophic cartilage and is expressed at later stage of cartilage differentiation, *i.e.*, in the initiation of bone formation. Therefore, the expression of Type X collagen is not preferable for tissues used for tissue regeneration. In the present invention, Type X collagen is not expressed, however, Type II collagen is expressed in the cartilage tissue produced. The cartilage tissue produced by the method of the present invention shows typical properties of cartilage tissue characterized by higher expression of aggrecan, and by safranin O staining (+).

In contrast, as discussed above, the three dimensional tissue mass developed with chondrocytes and stromal cells, as disclosed in Goodwin et al. shows no typical cartilage characteristics other than the expression of Type IV, IX, X collagens.

Further, Applicants submit that Schwarz et al. and Unsworth et al. do not make up for the deficiencies of Goodwin et al.

Therefore, Applicants submit that Goodwin et al. do not disclose the method of producing the cartilage tissue as recited in present Claim 1.

Accordingly, present Claim 1 is believed to overcome this rejection, and further, Claims 2, 3, 5 and 9 are believed to be patentable by virtue of their dependency from Claim 1. Withdrawal of this rejection is respectfully requested.

Claim Rejections – 35 U.S.C. § 103

(A) On page 9 of the Office Action, Claims 1-5, 8 and 9 remain rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Goodwin et al. and Schwarz et al., as applied to Claims 1-5 and 9.

Initially, Applicants submit that Claims 4 and 8 have been canceled thereby rendering the rejection moot for these claims.

Further, Applicants submit that Goodwin et al. do not disclose the method of producing the cartilage tissue as recited in present Claim 1, as discussed above, and that Schwarz et al. do not make up for the deficiencies of Goodwin et al. Thus, the cited references do not teach or suggest each and every element of the claimed invention and a *prima facie* case of obviousness has not been made. Accordingly, present Claim 1 is believed to overcome this rejection, and

further, Claims 2-3, 5 and 9 are believed to be patentable by virtue of their dependency from Claim 1.

Additionally, Applicants respectfully traverse the rejection for the following reasons.

The Office Action points to column 4, lines 20-25 of Goodwin et al., stating the problem of two-dimensional culture as the alleged motivation to use the method of three-dimensional culture by RWV.

However, present Claim 1 uses the two-dimensional culture and subculture as a preliminary process for three-dimensional culture. The efficiency of the three-dimensional culture by RWV is remarkably enhanced by the preliminary processes, *i.e.*, two-dimensional culture to confluence and subculture.

Under the confluent condition of the preliminary process, matrix production becomes more active than cell proliferation. Cartilage is generally a tissue containing fewer cells and having a rich extracellular matrix (for example, in the case of articular cartilage, the water content is 65-80%, Type II collagen is 10-20%; proteoglycan is 3-7%, and cartilage cell is 1%). In addition, the extracellular matrix is distributed among the cells and thus prevents tight aggregation of the cells, thereby allowing generation of cartilage tissue with elasticity and flexibility. In contrast, if two-dimensional culture and subculture have not been conducted, the cells would make a tight aggregate in three-dimensional culture using RWV and would generate cartilage tissue without elasticity and flexibility. Thus, in order to construct the cartilage tissue of the present invention, plenty of matrix proteins such as Type II collagen would needed. Accordingly, in order to construct the cartilage tissue of present Claim 1, it is required that "bone

marrow cells are two-dimensionally cultured to confluence, subcultured, and then cultured in a simulated microgravity environment."

Applicants submit that since Goodwin et al. teach that human bone marrow cell production decreases over time in two dimensional cultures, Goodwin et al. teach away from the invention in present Claim 1 and thus one of ordinary skill in the art would not have been motivated to combine the cited references to arrive at the invention in present Claim 1.

Withdrawal of the rejection is respectfully requested.

(B) On page 11 of the Office Action, Claims 1-6 and 9 remain rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Goodwin et al. and Schwarz et al. as applied to Claims 1-5 and 9 above, and further in view of Synthecon.

Initially, Applicants submit that Claim 4 has been canceled thereby rendering the rejection moot for this claim.

Further, Applicants submit that Goodwin et al. do not disclose the method of producing the cartilage tissue as recited in present Claim 1, as discussed above, and that Schwarz et al. and Synthecon do not make up for the deficiencies of Goodwin et al. Thus, the cited references do not teach or suggest each and every element of the claimed invention and a *prima facie* case of obviousness has not been made. Accordingly, present Claim 1 is believed to overcome this rejection, and further, Claims 2, 3, 5, 6 and 9 are believed to be patentable by virtue of their dependency from Claim 1.

Additionally, Applicants submit that the size of the RWV vessel as recited in Claim 6, which depends on the size of the culture required by the experimenter, is not a matter of routine

optimization. The inventors found that the best culturing conditions are conducted by seeding bone marrow cells at a density of 10^6 to 10^7 cells/cm^3 at a rotation speed of 8.5 to 25 rpm when a 5-cm RWV vessel is used. These conditions for the production of cartilage tissue is not routine because the conditions of conducting the culture must be such that the sinking speed of the seeded cells synchronizes with the rotation speed of the vessel, and the influence of the ground gravity imposed on the cells is minimized. See, page 8, lines 17-19 of the specification. Further, "[t]he rotation speed was frequently adjusted manually by visually inspecting the cell aggregate to maintain a stationary position in a vessel (time course of the rotation speed of an RWV is shown in Fig. 5). Bubbles would occur because of cellular respiration, and it would disturb the simulated microgravity environment. Thus, bubbles were frequently removed." See, page 9, line 29 bridging to page 10, line 5. This indicates that the cartilage tissue was obtained with great difficulties. Applicants submit that it would be a hindsight reconstruction of the claimed invention to assert that the cartilage tissue can be provided from the cited references through routine experimentation.

Withdrawal of the rejection is respectfully requested.

(C) On page 13 of the Office Action, Claims 1-5, 7 and 9 remain rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Goodwin et al. and Schwarz et al. as applied to Claims 1-5 and 9 above, and further in view of Yan et al. (U.S. 2002/0168763) and Simpson et al. (U.S. 2002/0090725).

Initially, Applicants submit that Claim 4 has been canceled thereby rendering the rejection moot for this claim.

Further, Applicants submit that Goodwin et al. do not disclose the method of producing the cartilage tissue as recited in present Claim 1, as discussed above, and that Schwarz et al., Yan et al., and Simpson et al. do not make up for the deficiencies of Goodwin et al. Thus, the cited references do not teach or suggest each and every element of the claimed invention and a *prima facie* case of obviousness has not been made. Accordingly, present Claim 1 is believed to overcome this rejection, and further, Claims 2, 3, 5, 7 and 9 are believed to be patentable by virtue of their dependency from Claim 1.

Finally, with respect to new Claims 10 and 11, Applicant submits that the requirements set forth therein further distinguish the claimed invention.

In regard to new Claim 10, it is noted that Goodwin et al. do not teach or suggest cartilage tissue having a major axis of over 1 cm.

Goodwin et al. states at column 5, lines 41-44 that "[t]he process for producing the normal mammalian tissue is particularly unique in that the resultant product is normal tissue of a size of 2 millimeters and larger. The size of the tissue cultured is significant because assembly of three dimensional masses of this size is not possible without the complex functional interrelationship of the normal mammalian cells." Tissues are three-dimensional structures, not two-dimensional having only a length and width. Particularly, although 1 cm is just five times that of 2 mm in terms of length, 1 cm is 125-times that of 2 mm in diameter, in terms of volume. Thus, Applicants submit that "2 millimeters and larger" cannot be considered to suggest 1 cm in diameter because a tissue having a diameter measurement of 2 mm (a volume of 0.004 cm^3) is

insufficient for clinical use (cartilage tissue regeneration). That is, a tissue having a diameter of 1 cm or more is required for clinical use (cartilage tissue regeneration).

Moreover, Figures 1 to 9 in Goodwin et al. show that the sizes of constructed tissues are at most about 1 mm in diameter. Therefore, Applicants assert that Goodwin et al. and the other cited references do not teach or suggest the tissue having a major axis of 1 cm or more as recited in new Claim 10.

In regard to new Claim 11, it is noted that Goodwin et al. do not teach or suggest adjusting the rotation speed of the simulated microgravity environment to synchronize with the sinking speed of the seeded cells.

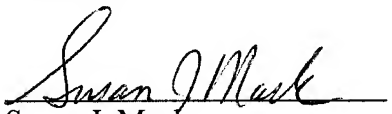
In the method described in Goodwin et al., the tissues (spheroids) in the RWV vessel are rotated in accordance with the rotation of the vessel and medium therein. See, column 8, lines 8-16. In contrast, in the method of the present invention, the tissues in the RWV vessel are balanced so as to maintain their relative positions. See, page 9, line 29 bridging to page 10, line 3 of the specification. This enables the production of large tissues with a major axis of over 1 cm in the method of the present invention. Therefore, Applicants assert that Goodwin et al. and the other cited references do not teach or suggest adjusting the rotation speed of the simulated microgravity environment to synchronize with the sinking speed of the seeded cells as recited in new Claim 11.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the

Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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